2015 BOTTLENOSE DOLPHIN (*Tursiops truncates*)
SAMPLE ANALYSIS PLAN
(RE: TEXAS CITY Y OIL SPILL NATURAL RESOURCE DAMAGE ASSESSMENT)

MARCH 18, 2015

This 2015 Sample Analysis Plan is for obtaining data for the Natural Resource Damage Assessment ("NRDA") associated with the Texas City Y ("TCY") oil spill. Each Party below reserves its rights to produce its own independent interpretations and analyses of any data collected pursuant to this Work Plan.

The Trustees have developed a preliminary conceptual model of the TCY release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the National Oceanic and Atmospheric Administration's ("NOAA") decision to pursue these sample analyses. By signing this Work Plan and agreeing to fund the work outlined, Kirby Marine, Ltd ("Kirby") is not necessarily endorsing the model or underlying assumptions that the trustees have developed, nor is it endorsing the conclusions in any Final Report produced.

This plan will be implemented consistent with existing Trustee regulations and policies.

All applicable state and federal permits must be obtained prior to conducting work.

APPROVED:

_____ _____

NOAA Trustee Representative: Date

_____ _____

Kirby Representative: Date

SCOPE AND OBJECTIVE:

NOAA proposes laboratory analysis of selectively targeted samples taken in accordance with the cooperatively funded 2014 Bottlenose Dolphin Collection Plan to assess whether bottlenose dolphins have been exposed and/or potentially injured by the TCY oil spill. NOAA requests funds in the amount of **$43,751** to cover the laboratory costs detailed below.

BACKGROUND:

On March 22, 2014, the 585-foot bulk carrier M/V *Summer Wind* collided with the oil tank-barge *Kirby* 27706 in Galveston Bay near Texas City, Texas, resulting in an oil spill of approximately 168,000 gallons of intermediate fuel oil (hereinafter, "the Incident" or "TCY spill"). Kirby has been identified as a Potentially Responsible Party for the TCY spill.

Intermediate fuel oil is viscous, sticky, and persistent. Only a relatively small amount is expected to evaporate within the first hours of a spill and/or mix into the water column. Though this type of oil can form thick slicks and be transported long distances by winds and currents, the majority of the TCY oil stranded on shorelines between Galveston and Matagorda Islands in Texas.

Oil exposure can have profound impacts on reproduction, health, and mortality of marine mammals (Lipscomb et al., 1993; Matkin et al., 2008; Mohr et al., 2008). NRDA work conducted in the northern Gulf of Mexico in the aftermath of the Deepwater Horizon oil spill is providing strong evidence for adverse impacts of oil exposure on the reproduction, health, and survival of marine mammals (Schwacke et al., 2013; Venn-Watson et al., in review). As such, NOAA believes it is necessary to assess the potential adverse impacts of bottlenose dolphins exposed to the TCY spill.

NOAA – as the designated lead agency for coordination of the Marine Mammal Stranding Network ("MMSN") under the Marine Mammal Protection Act ("MMPA") – has extensive expertise in the management and evaluation of marine mammals. This includes expertise in the historical compilation of databases relating to cause of death, state of health, and prevalence of disease among dolphins in the Gulf of Mexico and throughout the United States. NOAA will draw upon this expertise when evaluating baseline conditions corresponding to the TCY spill and discerning whether a measurable adverse impact has occurred.

The Trustees and Kirby have formalized a Memorandum of Agreement ("MOA") to provide the terms for a cooperative NRDA for the TCY spill. A "2014 Bottlenose Dolphin Sample Collection Plan" (the "2014 Collection Plan") was agreed to by the

Trustees and Kirby whereby funding for collection, sampling and storage of stranded bottlenose dolphin samples was provided under the MOA's "Advanced Funding" provisions. All samples proposed for analysis in this 2015 Sample Analysis Plan were collected under the 2014 Collection Plan. This 2015 Sample Analysis Plan proposes no new data collection from stranded animals.

Although NOAA essentially proposed this sample analysis plan as part of the 2014 Collection Plan, Kirby indicated it was not willing to fund laboratory sample analysis at that time. NOAA is willing to continue to discuss performing this 2015 Sample Analysis Plan cooperatively with Kirby, but NOAA will additionally pursue legal mechanisms to secure funding for this Plan if cooperative discussions are not fruitful within a timely manner – as indicated in NOAA's Presentment of Demand Letter that accompanied this Plan's presentation to Kirby.

NOAA AND MARINE MAMMAL STRANDING DATA:

The MMSN was formalized as part of the 1992 Amendments to the MMPA. NOAA's National Marine Fisheries Service ("NMFS") was designated as the lead national agency to establish the MMSN Program, and to coordinate responses in United States waters for all cetaceans and pinnipeds (except walrus). Volunteer participants in the MMSN exist in all coastal states and territories where they are available to respond to marine mammal strandings. Such responses are authorized – by Section 112c "Stranding Agreements" – by one of NOAA's six NMFS Regional Offices, or under Section 109h of the MMPA for Federal, State, or local government officials operating in an official capacity. In the Gulf of Mexico, including Texas, NOAA NMFS's Southeast Regional staff coordinates MMSN response.

Basic information ("Level A data") regarding marine mammal strandings (location, date, time) in the United States is collected by a national MMSN and is recorded on nationally standardized forms (OMB No. 0648-0178, NOAA Form 89-864). Level A data are entered into a national NOAA database that is publicly available. A goal of NOAA's longstanding, routine data collection on marine mammal strandings is identification of baseline health conditions and standards against which unusual events or episodes may be compared. Analysis of stranding data can also indicate certain life stages – or life events such as pregnancy – when dolphins are most sensitive to environmental disruption.

NOAA is mandated by the MMPA (at 16 USC s. 1421h) to investigate "Unusual Mortality Events" ("UMEs") in marine mammal communities. A formal process is directed by the MMPA, by which NOAA evaluates baseline conditions, reviews historical data and consults with marine mammal experts in determining the likely

causes of such UMEs. For example, the 1992 Matagorda Bay UME, and the 1994, 2008 and 2010 Texas-wide UMEs – among others in the Gulf of Mexico – provide extensive data on the baseline conditions and common causes of death for dolphin communities in the TCY spill area.

Although NOAA does not propose labor costs to analyze stranding data in this 2015 Sample Analysis Plan, it is important to note that sample results from this 2015 Sample Analysis Plan will be interpreted by NOAA experts familiar with both baseline conditions in the TCY area and known signals of episodic or unusual events in dolphin communities in Texas. Counts, demographics, and spatio-temporal distribution of stranded dead animals following the TCY spill will be evaluated in a historical context to assist in quantifying mortality potentially attributable to the TCY spill. Labor costs to perform these evaluations are projected to be covered under the Cooperative MOA signed by the Responsible Party. Results of these stranding data evaluations will inform the interpretation included in NOAA's Final TCY Bottlenose Dolphin Sample Analysis Report.

2014 SAMPLE COLLECTION PLAN PROTOCOLS:

Six Month Timeframe:

As agreed in the 2014 Collection Plan, NOAA requested[1] that the Texas MMSN ("TXMMSN") follow sampling, necropsy, handling, and documentation protocols in a targeted area from the date of the spill through six months following the spill (March 22, 2014 through September 22, 2014). NOAA believes that six months following a spill in Texas is a reasonable time frame for assessing whether acute impacts from the TCY spill have occurred.

Targeted Sampling of Stranded Animals:

Bottlenose dolphins in the nearshore waters of Texas experience a spring calving peak from February through April. Therefore, at the time of the TCY oil spill

[1] These MMSN organizations/participants receive no consistent financial support from the federal government for their activities. Thus the ability of the MMSN to respond to and investigate strandings can vary by organization, by level of training, by level of other funding, and by year. Participants in the MMSN may apply for Prescott grants in annual competitions or through emergency funds, which do provide limited support for stranding response activities to some MMSN organizations. In every case, Stranding Network Participants must consider the practicability of collection and samples, the availability of Stranding Network Resources, and the health and safety of Stranding Network participants when deciding whether samples can be collected according to NOAA requests.

(March 22, 2014), there would have been numerous later term pregnant females and/or newborn bottlenose dolphin calves in the nearshore and estuarine waters of Texas. These animals are likely to be the most susceptible dolphins to oil exposure and oil effects, since they spend the most time at the surface of the water where they can be exposed through direct inhalation or aspiration of oil.

In order to specifically target these most-at-risk dolphins, the 2014 Collection Plan requested that the TXMMSN selectively collect, sample and store carcasses from the following three types of stranded bottlenose dolphins:

- Pregnant females;
- Perinatal or young of the year; or
- Visibly oiled dolphins.

NOAA further requested that the TXMMSN selectively collect, sample and store carcasses and samples differently than their normal operating procedures. This resulted in additional NRDA-specific costs. An estimated budget of those additional costs was included in the 2014 Sample Collection Plan, and was funded through the Cooperative MOA "Advanced Funding" provisions.

Although the TXMMSN will continue to collect data in Texas, no further collection of stranded dolphin samples for TCY assessment purposes is proposed under either the 2014 Sample Collection Plan or this 2015 Sample Analysis Plan.

2014 SAMPLE COLLECTION PLAN RESULTS:

In the entire state of Texas, a total of 57 bottlenose dolphins were recorded by TXMMSN from the start of the oil spill for a period of 6 months following the spill (March 22, 2014 - September 22, 2014). Stranding demographics and temporal and spatial data were collected by the TXMMSN using standardized Level A data records for all 57 stranded dolphins.

Of the 57 stranded dolphins collected, 28 fell into the categories specifically targeted for collection, storage and sampling under the 2014 Sample Collection Plan. That is, 28 of the 57 stranded dolphins were: 1) pregnant, 2) perinatal or "young of the year", and/or 3) visibly oiled.

Table 1: Stranded Dolphins in Targeted Sampling Categories (March 22, 2014 – September 22, 2014)

Category	Number of Dolphins
Pregnant	0
Perinatal or "young of the year"	25
Visibly Oiled	3*

* 1 was young of the year and 2 were adults

Challenges of working with tissues from stranded animals include the ability to quickly obtain high quality samples (as fresh as possible) and ensuring proper handling and storage of these samples. As a result, the 2014 Collection Plan targeted samples from fresh and moderately decomposed stranded dolphins. In an effort to generate only reasonable damage assessment costs corresponding to a spill of the magnitude of the TCY incident, NOAA is currently proposing that only 4 of the 28 dolphins – i.e. those with the most favorable decomposition states – be slated for laboratory testing at this time. NOAA believes that targeting the 4 perinatal ("young of the year") dolphin in the least decomposed state that were found within the footprint of the TCY spill within 6 months of the TCY spill provides a targeted and cost effective approach to assessment of this TCY spill injury assessment category. Table 2 lists these 4 stranded dolphins targeted for further laboratory analysis under this 2015 Sample Analysis Plan.

Table 2: Stranded Dolphins that Fit Sampling Criteria and Decomposition State Level

Field Number	Strand Date	Total Length (cm)
GA1894	2014-Mar-28	175 *(183 at necropsy)*
GA1905	2014-Mar-30	101
GA1915	2014-Apr-02	187
PA1049	2014-Jun-06	99 **

** Caudal peduncle and tail flukes cut off due to shark bite

* GA = stranded in or near Galveston Bay, TX

* PA = stranded in or near Port Aransas, TX

PROPOSED LABORATORY ANALYSES:

The Trustees have developed a preliminary conceptual model of the TCY spill, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the Trustees' decision to pursue the studies outlined below. This 2015 Bottlenose Dolphin Sample Analysis Plan proposes analyses regarding three categories of laboratory evaluation: 1) polycyclic aromatic hydrocarbon (PAH) analysis; 2) histopathologic evaluation; and 3) testing to "rule in-or-out" other common causes of dolphin mortality. All proposed analyses will be conducted within normally accepted or required holding times for the various sample types and associated analyses.

Proposed PAH Analysis:

The purpose of the TCY NRDA is to determine whether natural resources were exposed to oil and suffered any measurable adverse impact as a result. The PAH analysis proposed in this Plan will provide Trustees with information to determine: 1) whether suspect oil and tissue samples actually contain PAHs; 2) whether PAHs (if identified) are consistent with the "signature" of the oil released in the TCY incident; and 3) the concentration of PAHs, where identified.

Swabs and/or tissue samples were collected and will be evaluated to determine exposure to PAHs and source oil fingerprinting, where possible. It is important to note that samples from PA1049 are not proposed for PAH analysis at this time since PA1049 stranded 2.5 months after the spill (June 2, 2014). Where possible, NOAA proposes that samples be fingerprinted to identify the source of the oil, including where scientifically reasonable methods exist for characterizing oil found in dolphin tissue.

Proposed Swab Analysis for PAHs:

In the event that obvious or suspect visible oil was found on any of the 3 dolphins, the suspect material was sampled using a TCY NRDA swabbing protocol. NOAA proposes to analyze the swabs for Total Petroleum Hydrocarbons (TPH) to confirm that petroleum is present. If petroleum presence is confirmed, samples will be analyzed for "fingerprinting", with comparison to a known source sample of the TCY oil spilled.

Proposed Tissue Analysis for PAHs:

Where an animal was believed to have been contaminated with suspect oil, a sample of their potentially affected tissue(s) was collected for PAH analysis. If detected and where possible, NOAA will attempt to characterize the PAHs in the tissue(s) using existing scientifically reasonable methods. It is notable that one dolphin (GA1894) was visibly externally oiled and an unknown black/dark brown substance was detected in its esophagus during necropsy. Another dolphin (GA1905) was noted as having an unidentified acrid chemical smell during necropsy.

It is possible that dolphins may have inhaled oil or volatile compounds as a result of the TCY spill. This is particularly true given that the 2014 Sample Collection Plan targeted dolphins likely spend the most time at the surface of the water, where they can be exposed through direct inhalation or aspiration of oil. PAH analysis of targeted lung samples is therefore proposed.

Dolphins may also ingest oil during exposure; targeted analysis of stomach contents for the presence of PAHs is also therefore proposed.

Proposed Histopathological Analysis:

Histopathological analysis consists of a standard, targeted set of procedures performed by a recognized professional to identify lesions, diseases, and cause of death. Histopathological analysis by a certified professional is proposed to determine whether characteristics of these 4 targeted dolphins' pathology is consistent with oil exposure, even if external oiling was not immediately evident at the time of sample collection.

To assess potential contributing factors and causes of death, tissue samples from major organs for each dolphin were collected by the TXMMSN and/or during necropsy and will be histologically evaluated (Venn-Watson et al., in review). Each of the 4 dolphins in the targeted subset is proposed for histological analysis.

Although gross necropsies were conducted at the time of stranding, in many cases cause of death can often best be determined by a microscopic review of tissues for signs of disease. Therefore, histopathological analyses will be performed to microscopically examine the tissues from the 4 targeted dolphins and secure a professional judgement as to likely cause of death.

Proposed Differential Analysis ("Rule Out"):

Analysis of historical marine mammal strandings data assist in dolphin injury assessment by providing evidence of both oil and non-oil stressors that are known to affect bottlenose dolphin populations in Texas. Non-oil factors may include disease, human and fisheries interactions, cold events and numerous other environmental

factors (Litz et al., 2014). NOAA's extensive experience with evaluation of UMEs in the Gulf of Mexico, and Texas in particular, generally provides a good baseline dataset to predict common causes of mortality for bottlenose dolphins that may be used to "rule out" the TCY incident as a factor in dolphin deaths following the TCY spill.

NOAA proposes the following "rule out" set of laboratory analyses as part of this 2015 Sample Analysis Plan:

> Biotoxin tests will be conducted to determine if a dolphin may have been exposed to a toxic algal bloom that was occurring in Texas at the time of the spill, which could have contributed to its cause of death.
>
> Feces and stomach content samples will be analyzed using methods previously described and commonly employed (Maucher et al., 2007; Fire et al., 2008; Fire et al., 2011; Venn-Watson et al., in review) to determine if common disease symptoms or causes of mortality are indicated.
>
> Exposure to the *Brucella* bacteria may contribute to a bottlenose dolphin's cause of death (Guzman-Verri et al., 2012; Litz et al., 2014). A *Brucella* PCR assay will be used to test for *Brucella* in multiple tissues for each dolphin according to published protocols (Ficht et al., 1996; Venn-Watson et al., in review).

BUDGET:

Trustees acknowledge that the figure provided is an estimate and actual costs may prove to be higher. In that case, the trustees will make a good faith effort to notify Kirby in advance of any such increased costs.

Table 3: Estimated budget for bottlenose dolphin sample analysis.

Tissue Analytical Costs **TIER 1 Analyses:**		
Facility	**Test**	**Cost per Animal (4 animals)**
Univ of Illinois	Histo & Dx (micro culture, fungal screen, special stains, bacterial screens, IHC)	$500
NCSU	Histo processing	$150
	TOTAL	**$2,600**
Facility	**Test**	**Cost per Sample (13 samples)**
NewFields	PAH analysis	$34,625
	TOTAL	**$34,625**
TIER 2 Analyses:		
Facility	**Test**	**Cost per Sample (6 samples)**
NOS	Biotoxins	$550
	TOTAL	**$3,300**
Facility	**Test**	**Cost per Sample (2 samples/dolphin)**
Univ of Illinois	*Brucella* PCR testing	$880
NVSL/USDA	*Brucella* Culture	$320
	TOTAL	**$1,200**
½ day of tech time at NMFS SEFSC to open stomachs & subsample for PAH & Biotoxins		

(note $37.71/hr x 4 hrs + 16% overhead)			
		TOTAL	$176
TXMMSN Costs			
Carcass disposal	4 carcasses		$35/carcass
		TOTAL	$140
Additional Costs			
Sample Shipping Costs (via FedEx)			$1,000
IEc Contract Overhead			$710
		TOTAL	$1,710
TOTAL COSTS FOR DOLPHIN ASSESSMENT IN TX			**$43,751**

SAMPLE AND DATA HANDLING AND SHARING:

Sample and Data Handling:

Samples and associated data were collected under the 2014 Collection Plan. The data are being tracked, organized, and are undergoing Quality Assurance/Quality Control ("QA/QC") by NOAA. These data, along with associated documentation such as validation reports, necropsy reports and chain-of-custody ("COC") forms are being maintained by NOAA and will ultimately be distributed to Trustee and Kirby representatives through NRDA and cooperative databases.

TCY NRDA COC procedures were and will continue to be observed for all targeted samples under the 2014 Collection Plan. All samples will be transferred for analysis in accordance with appropriate COC forms and legal permitting regimes under the MMPA. All laboratory data will be collected, managed and stored in a secure facility under Trustee control in accordance with NOAA's written Standard Operating Procedures ("SOPs"). All proposed analyses will be conducted within normally accepted or required holding times for the various sample types and associated analyses.

All materials associated with the collection or analysis of samples – except those consumed as a consequence of the applicable sampling or analytical process, or where practicability or human health and safety concerns prevents it – will be retained unless and until NOAA approval is given for their disposal. Any samples collected under the 2014 Collection Plan, but not analyzed under this 2015 Sample Analysis Plan, will remain archived for potential future analysis or returned to the TXMMSN. The Trustees will prepare an addendum to this Plan if they determine additional samples need to be analyzed.

Data Sharing:

All samples analyzed pursuant to this Plan will be submitted to laboratories that are operated in a manner that is consistent with NOAA permits, regulations and guidelines. Under this Plan, each laboratory shall deliver raw data generated as part of this work plan to NOAA. Thereafter, NOAA will validate and perform QA/QC procedures on the data, including working with the respective laboratory to achieve this result, after which time the validated QA/QC data shall be made available simultaneously to all Trustees and Kirby. Although NOAA may – at its discretion or through a cooperatively devised formal sharing mechanism – share raw, unvalidated data with Kirby and/or its co-Trustees, only the validated/QA/QC data set released by NOAA shall be considered the final data set. Should any party show a critical operational need for data prior to validation and QA/QC, any released data, if so released, will be clearly marked "preliminary/unvalidated" and will be made available equally to all Trustees and to Kirby under a negotiated confidentiality agreement.

The implementation of this Plan will occur cooperatively with NOAA's co-Trustees, results of these analyses will be shared with co-Trustees, and interpretation and final conclusions of this Plan will be coordinated with co-Trustees. NOAA continues to actively work with its co-Trustees to ensure there is no duplication of efforts in relation to this assessment of potential dolphin injury associated with the TCY spill.

DELIVERABLES AND PROJECTED TIMETABLE:

Laboratory analyses are performed as samples are compiled and shipped to the lab. Lab reports are provided to NOAA as results are obtained. A final conclusory "Bottlenose Dolphin Analysis Report" – with indications of NOAA's estimate of the assessed injury to dolphins as a result of the TCY spill -- will be produced as a product of this effort. NOAA labor to complete final conclusions will be incorporated into the labor costs reimbursement mechanisms outlined in the Cooperative MOA. NOAA projects that the final Report will be available within eight months of approval of this Plan.

RESPONSIBLE PARTY INVOLVEMENT:

NOAA has not yet been able to secure cooperative agreement from Kirby to fund or participate in this Plan.

PRINCIPAL INVESTIGATORS:

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